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Patent- og Varemærkestyrelsen
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PATENT- OG VAREMÆRKESTYRELSEN

Opfindelsen angår et antimikrobielt præparat til lokal anvendelse på hud og slimhinder.

Hud- og slimhindeinfektioner forårsages af såvel Gram positive som Gram negative bakterier.

Antimikrobielle midler anvendes lokalt på slimhinder og sår. Formålet med en lokal behandling kan være at supplere en systemisk behandling med en lokal tilførsel hvor forholdene tillader det, og hvor man ellers ikke kan opnå tilstrækkelige koncentrationer i lokale infektionsfoci ved en systemisk behandling alene, f.eks. ved behandling af panaritium.

Lokal behandling med antibiotika i creme eller salveform anvendes også ved overfladiske infektioner. Ved at vælge denne behandlingsform, frem for den systemiske antibiotika behandling, undgår man de bivirkninger der kan været forbundet med den systemiske behandling.

Desuden finder en række forskellige desinfektionsmidler anvendelse ved behandlingen af infektiøse hudlæsioner.
Begge grupper af antimikrobielle midler til lokal brug, både antibiotikagruppen og desinfektionsgruppen, er behæftede med væsentlige ulemper.

Antibiotika
Ved systemisk behandling med antibiotika er der altid en risiko for sensibilisering af patienten, men denne risiko er væsentligt forøget ved lokal brug af det pågældende antibiotikum.
Dette forhold gør at der er stor tilbageholdenhed med at anvende antibiotika lokalt, i form af cremer eller salver, ved banale hud eller slimhinde infektioner, idet en sensibilisering med et antibiotikum eller flere antibiotika vil indskrænke patientens behandlingsmuligheder hvis en alvorlig infektion senere i livet skulle kræve en systemisk antibiotikabehandling.
Desuden indebærer lokal behandling med antibiotika en øget risiko for udvikling og selektion af resistente bakterier, især langtidsbehandling af hudinfektioner er i så henseende meget betænkeligt.

Desinfektionsmidler
Desinfektionsmidler til behandling af lokaliserede eller overfladiske infektioner er alle at betragte som alment cel-

lebeskadigende midler. De mest anvendte er iodofor, chlorhexidin, kvaternære ammoniumforbindelser, brintperoxid og syrer.

Lysozym

I 1922 opdagede bakteriologen Alexander Flemming et hidtil ukendt enzym som han gav navnet lysozym på grund af dets evne til at lysere en række forskellige bakterier.

Lysozym forekommer i mange biologiske væsker f.eks. æggehvide, mælk, blod og tårer. Alexander Flemming stillede store forhåbninger til lysozym som terapeutikum ved infektionssygdomme, men det viste sig hurtigt at der var ingen effekt af lysozym overfor de allerfleste patogene bakterier.

Det forhold at lysozym virker på nogle bakterier og ikke på andre skyldes forskelle i bakteriernes ydre membran. Det er således kendt at Gram positive bakterier lyseres af lysozym, men at Gram negative bakterier ikke påvirkes af lysozym.

Forklaringen på dette fænomen er, at det yderste lag på Gram positive bakteriers cellemembran består af peptidoglycan (fig 1), som umiddelbart kan nedbrydes af lysozym, hvorved bakterier går til grunde (fig. 3).

Det forholder sig anderledes med Gram negative bakterier. Her består det yderste cellemembranlag af lipopolysaccharid og først derefter et peptidoglycanlag (fig. 2). Da lysozym ikke er i stand til at spalte lipopolysaccharidlaget (fig. 4) vil det sige, at dette yderste lag først må fjernes eller perforeres inden lysozym kan ødelægge Gram negative bakterier.

Der har gennem tiderne været udfoldet mange bestræbelser for at klare dette problem uden at opnå tilfredsstillende resultater. Der har været forsøgt at kombinere lysozym med EDTA, polyphosphat, kaliumsorbat og meget andet, men ingen af disse metoder har med fordel kunnet anvendes til udformning af præparater til brug på hud og slimhinder og har desuden ikke haft en generel virkning på den brede vifte af Gram negative bakterier.

Immunglobuliner (gammaglobuliner, antistoffer).

I 1890 offentliggjorde Emil von Behring et videnskabeligt arbejde om behandling af tetanus med antitoksin.

Senere studier har vist at antitoksin var immunglobuliner og dette gav startskuddet til passiv immunterapi, hvor man profylaktisk injicerer immunglobuliner intramuskulært eller subkutant, mod f.eks. botulisme, difteritis, hugormebid og slangebid. Disse immunglobuliner stammer alle fra immunise-

rede dyr og er rettede mod toksiner. Desuden anvendes immunglobuliner af human oprindelse til profylakse mod tetanus og hepatitis B.

Det er velkendt at immunglobuliner spiller en rolle ved bekæmpelse af mikrobielle infektioner. For det første binder specifikke immunglobuliner sig til bakterieoverfladen og derved opnås en opsonisering der gør den pågældende bakterie mere attraktiv for fagocytterne. Det er ligeledes velkendt at immunglobuliner ikke er i stand til at aflive bakterier ved egen hjælp. Der kræves tilstedeværelse af et intakt komplementsystem, som aktiveres af immunglobulinerne for at kunne dræbe bakterier.

Dette forhold, at immunglobuliner er afhængige af komplement eller fagocytter i kampen mod bakterier, gør at immunglobuliner kun anses for at have effekt når det anvendes som injektionspræparat og dermed blandes op i serum og vævsvæske hvor der altid er rigeligt med komplement tilstede. Denne opfattelse støttes af den kendsgerning, at immunglobuliner ikke har nogen effekt overfor bakterier under in vitro forhold.

Der er beskrevet præparater til lokal anvendelse indeholdende blandt andet immunglobuliner og lysozym (EP offentliggørelsesskrift nr 1068071 og US patentskrift nr 4734279). Disse præparater har uden tvivl effekt overfor Gram positive bakterier på grund af indholdet af lysozym, men Gram negative bakterier påvirkes ikke af lysozym og heller ikke af lysozym i kombination med native immunglobuliner ved lokal applikation. Det er almindeligt kendt at bakterier er udstyret med proteolytiske enzymer der nedbryder immunglobuliner. Nogle bakterieenzymer har IgA1 som specifikt substrat, de såkaldte post-prolin endopeptidaser. Disse forhold er udførligt beskrevet af Mogens Kilian et al i APMIS 104: 321-338, 1996. IgG molekylet, som ikke findes på slimhinder, er meget følsomt overfor alle mulige proteaser og inaktiveres efter få minutter i de fleste bakteriesuspensioner.

Gentagne forsøg in vitro har således også vist manglende effekt af kombinationen lysozym og nativt immunglobulin på Gram negative bakterier.

Formålet med nærværende opfindelse er at anvise et antimikrobielt præparat til lokal anvendelse uden bivirkninger og uden de ulemper som er forbundet med antibiotika og desinfektionsmidler.

Dette opnås ifølge opfindelsen ved at præparatet omfatter et system bestående af en kombination af lysozym og glycosyleret immunglobulin.

Fordelen ved opfindelsen er at den har løst problemet for lysozym med at penetrere den ydre lipopolysaccharid membran på Gram negative bakterier og dermed skabt mulighed for nedbrydning af peptidoglycan membranen med bakteriolyse til følge (fig. 5).

Opfindelsen er baseret på in vitro forsøg med Gram negative bakterier og de korresponderende glycosylerede immunglobuliner rettet mod bakteriens overflade antigen.
Det viste sig at når bakterievæggens overflade antigen blev påhæftet et glycosyleret immunglobulin, medførte dette, at bakterierne kunne dræbes med lysozym.
Den mulige forklaring på dette fænomen er, at det glycosylerede immunglobulinmolekyle (antistoffet) binder sig til bakterievæggens overflade antigen determinant, hvorved bakterievæggens overfladeegenskaber ændres i en sådan grad at lysozym aktivitet på det underliggende lag af peptidoglycan muliggøres.
Det er en forudsætning at de anvendte immunglobuliner er intakte og modstandsdygtige overfor bakterieproteaser. Dette opnås ved glycosylering af immunglobulinernes Fc fragment.
10 g immunglobuliner poolet 77,0% IgG, 7,6% IgA og 15,4% IgM opløses i 25 ml 1M glucoseopløsning og inkuberes ved 45°C.
Glucosen vil under inkubationen etablere kovalente bindinger, især til IgG molekylets Fc fragment, hvilket kan kontrolleres ved påvisning af tab af komplement bindingsevne.
De glycosylerede immunglobuliners agglutinationsevne er derimod upåvirket.
En tilsvarende glucosylering er observeret in vivo hos dårligt insulinregulerede diabetes patienter. Et sådant glycosyleret immunglobulin er ekstremt modstandsdygtigt overfor såvel pankreas som bakterielle proteaser.

I laboratorieforsøg er der anvendt ikke nærmere typebestemte Gram negative stave og Gram negative kokker. Det anvendte lysozym er udvundet af hønse æggehvide, og immunglobulinerne er isoleret fra bovint mælk og colostrum.

Forsøg 1a
Bakteriesuspension (100.000 kim/ml) inkuberes 1/2 time med 5 mg/ml lysozym ved 37°C. Efterfølgende dyrkning på agarplade viste ingen bakteriedrab.

Forsøg 1b
Bakteriesuspension (100.000 kim/ml) inkuberes 1/2 time med 5 mg/ml lysozym + 40 µg/ml agglutinerende native antistoffer ved 37°C. Efterfølgende dyrkning på agarplade viste ingen bakteriedrab.

Forsøg 1c
Bakteriesuspension (100.000 kim/ml) inkuberes 1/2 time med 5 mg/ml lysozym + 40 µg/ml agglutinerende glycosylerede antistoffer ved 37°C. Efterfølgende dyrkning på agarplade viste 100% bakteriedrab.

Forsøg 2a
Bakteriesuspension bestående af 2 forskellige Gram negative bakterier inkuberes 1/2 time ved 37°C med 5 mg/ml lysozym + 40 µg/ml agglutinerende native antistoffer med specificitet overfor den ene bakterie. Efterfølgende dyrkning på agarplade viste ingen drab af bakterier.

Forsøg 2b
Bakteriesuspension bestående af 2 forskellige Gram negative bakterier inkuberes 1/2 time ved 37°C med 5 mg/ml lysozym + 40 µg/ml agglutinerende glycosylerede antistoffer med specificitet overfor den ene bakterie. Efterfølgende dyrkning på agarplade viste 100% drab af de bakterier som antistofferne var rettet mod og ingen påvirkning af de bakterier som ikke agglutinerede med de anvendte antistoffer.

For at tilnærme in vitro forsøgene til in vivo forhold var bakteriesuspensionen vækstbouillon, således at bakteriernes exogene enzymer var indeholdt.

Figurfortegnelse

Figur 1:
Skematisk tegning af Gram positiv bakteries cellevæg.

Figur 2:
Skematisk tegning af Gram negativ bakteries cellevæg.

Figur 3:
Diagram over Gram positiv bakteries lysering med lysozym.

Figur 4:
Diagram der illustrerer at Gram negative bakterier ikke påvirkes af lysozym alene.

Figur 5:
Diagram over Gram negativ bakteries lysering med glycosyleret immunglobulin og lysozym ifølge opfindelsen.

Eksempler på præparater der omfatter systemet ifølge opfindelsen.

1) Præparat mod hudinfektioner (gel)

Lysozym	0,5%
Glycosylerede immunglobuliner (poolet IgM, IgG, IgA)	0,4%
Resingel	99,1%
	100,0%

2) Præparat mod hudinfektioner (creme)

Lysozym	0,5%
Glycosylerede immunglobuliner (poolet IgM, IgG, IgA)	0,4%
Cremegrundlag	99,1%
	100,0%

3) Præparat mod hudinfektioner (til fugtighedsservietter)

Lysozym	0,5%
Glycosylerede immunglobuliner	0,4%
Pebermynteolie	0,1%
Sterilt vand	99,0%
	100,0%

3) Sugetablet mod infektioner i mund, hals og svælg

Lysozym	0,5%
Glycosylerede immunglobuliner	0,4%
Smagsstoffer og tablethjælpestoffer	99,1%
	100,0%

4) Tyggetablet mod infektioner i mave-tarm kanalen

Lysozym	0,5%
Glycosylerede immunglobuliner	1,2%
Smagsstoffer og tablethjælpestoffer	98,3%
	100,0%

Patentkrav

1. Antimikrobielt præparat til lokal anvendelse på hud og slimhinder kendetegnet ved at præparatet omfatter et system bestående af en kombination af lysozym og glycosylerede immunglobuliner med affinitet til Gram negative bakterier.

2. Antimikrobielt præparat ifølge krav 1 kendetegnet ved at de glycosylerede immunglobuliner er af mono- eller polyklonal oprindelse.

3. Antimikrobielt præparat ifølge krav 2 kendetegnet ved at de glycosylerede immunglobuliner er af enten klasserne IgM, IgG, IgA eller dimer IgA.

4. Antimikrobielt præparat ifølge krav 1 kendetegnet ved at immunglobulinerne har oprindelse i en biologisk væske såsom mælk, valle, blod, plasma eller serum.

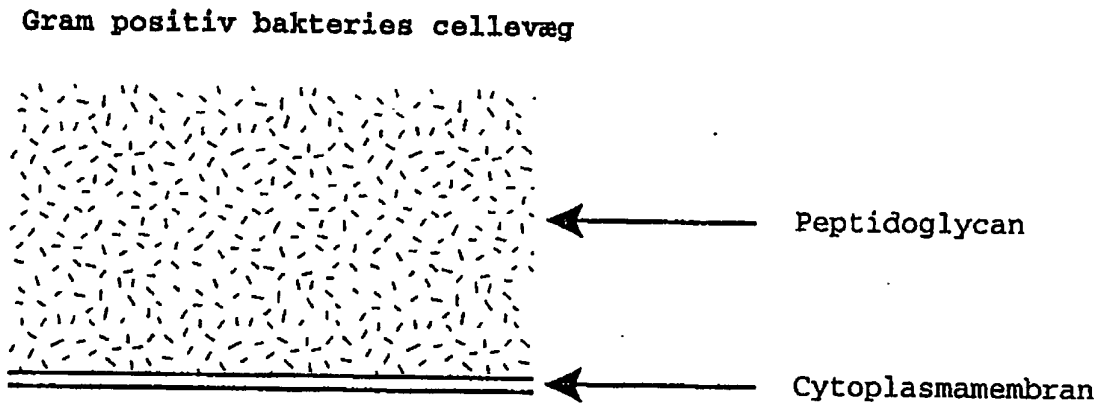
5. Antimikrobielt præparat ifølge krav 1 kendetegnet ved at lysozymet er enten nativt eller konjugeret.

Sammendrag

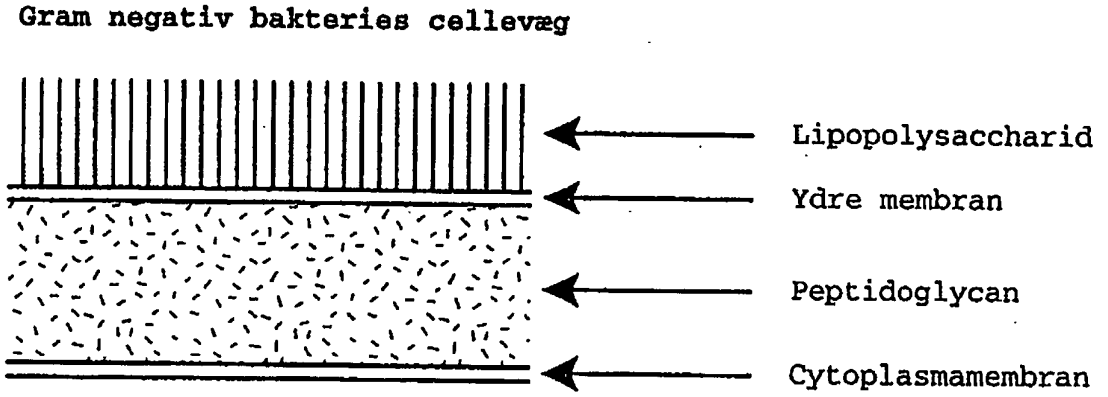
Et antimikrobielt præparat til lokal anvendelse på hud og slimhinder, bestående af en kombination af lysozym og glycosylerede immunglobuliner med affinitet til Gram negative bakterier.

Lysozym kan lysere Gram positive bakterier ved at nedbryde bakteriecellevæggens peptidoglycan (fig. 3). Gram negative bakteriers peptidoglycan er beskyttet mod påvirkning af lysozym, med et lipopolysaccharid lag (fig. 4). Når protease modstandsdygtige glycosylerede immunglobuliner binder sig til de Gram negative bakteriers cellevæg, sker der en sådan ændring af overfladens lipopolysaccharid lag, at lysozym er i stand til at nedbryde det underliggende peptidoglycan lag, med bakteriolyse til følge (fig. 5).

Figur 1

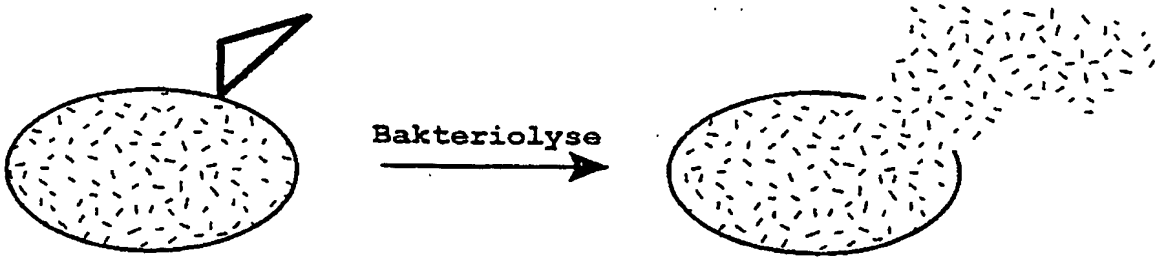


Figur 2



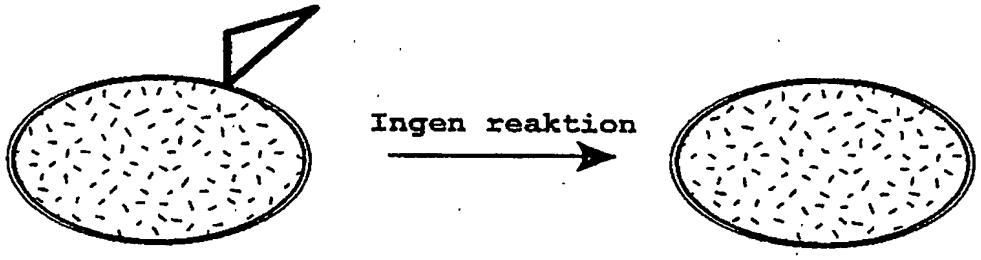
Figur 3

Gram positiv bakterie lyseres med lysozym



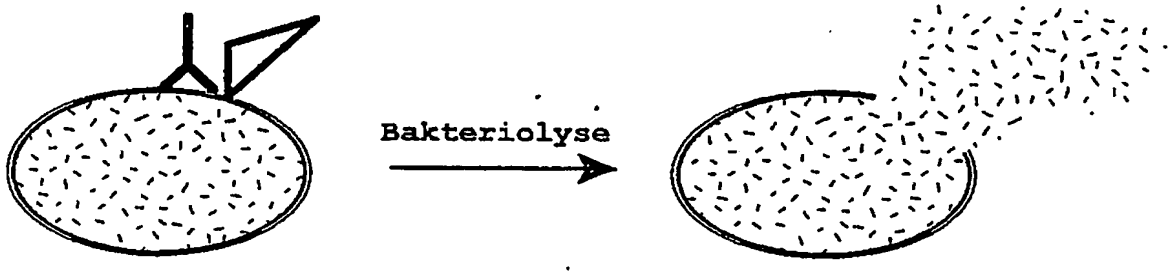
Figur 4

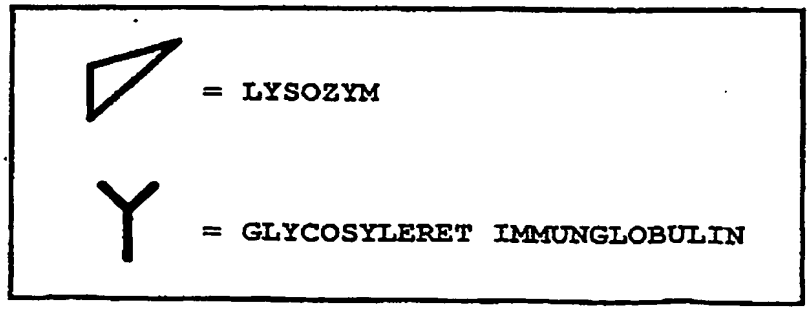
Gram negativ bakterie kan ikke lyseres med lysozym alene



Figur 5

Gram negativ bakterie lyseres med glycosyleret immunglobulin og lysozym





Antimicrobial composition for local use on mucosal membranes and skin

The invention relates to an antimicrobial composition for local use on mucosal membranes and skin.

Infections of the mucosal membranes and skin infections are caused by both Gram positive and Gram negative bacteria.

Antimicrobial agents are used for local use on mucosal membranes and for local use of lesions to the skin. The purpose of a local treatment may be to supplement a systemic treatment by local use in suitable cases where adequate concentrations of the antimicrobial agent cannot be achieved in local foci of infection by systemic treatment alone, e.g. in the treatment of paronychion.

Local treatment using antibiotics in the form of a cream or an ointment is also conducted in case of superficial infections. By choosing such a treatment in stead of a systemic treatment with antibiotics a number of side-effects that may be associated with systemic treatment can be avoided.

Furthermore, a number of diverse disinfectants are used in the treatment of infected lesions to the skin. Both groups of antimicrobial agents for local use, the group of antibiotics and the group of disinfectants, suffer from substantial disadvantages.

Antibiotics

By systemic treatment using antibiotics a risk of sensitization of the patient is always present but such a risk is substantially increased by local use of the antibiotic in question.
For this reason one is often reluctant in cases of trivial infections of mucosal membranes and skin to locally use antibiotics in form of creams or ointments as sensitization caused by one or more antibiotics will decrease the possibility of treating a patient should a serious infection occur later in life.
Furthermore, local treatment using antibiotic implicates an increased risk of the development and selection of resistant bacteria, in this respect particularly long-term treatment of skin infections is alarming.

Disinfectants

Disinfectants for the treatment of localised or superficial infections are collectively to be recognized as general cell damaging agents. The most widely used disinfectants include iodophor, chlorhexidine, quaternary ammonium compounds, hydrogen peroxide and acids.

Lysozyme

In 1922 the bacteriologist Alexander Fleming discovered a hitherto unknown enzyme which he termed lysozyme due to its ability to lyse a number of different bacteria.

Lysozyme is present in a number of biological fluids such as egg white, milk, blood and tears. Alexander Fleming had great expectations for lysozyme as a therapeutic for treatment of infectious diseases but soon it became clear that no effect of lysozyme against the most common pathogenic bacteria could be detected

The fact that lysozyme is functional against some bacteria and not against other types of bacteria is due to differences in the outer membrane of bacteria. It is well known in the art that Gram positive bacteria are lyzed in the presence of lysozyme whereas Gram negative bacteria are not affected by lysozyme.

The reason for this is that the outer layer of the cell membrane of Gram positive bacteria consists of peptidoglycan (Fig. 1) which is readily degraded by lysozyme which causes the bacteria to die (Fig. 3).

The situation for Gram negative bacteria is different from that of Gram positive bacteria. In Gram negative bacteria the outer layer of the cell membrane consists of lipopolysaccharide and only after that a layer of peptidoglycan (Fig. 2). As lysozyme is not able to cleave the layer of lipopolysachharide (Fig. 4) it means that this outer layer has to be removed or to be perforated before the lysozyme can destroy Gram negative bacteria.

Through the years a number of efforts have been made in order to solve this problem without achieving satisfying results. One has tried to combine lysozyme and EDTA, polysorbate, potassium sorbate amongst other but none of these methods have been suitable for the design of a composition for use on mucosal membranes and skin, moreover, a general effect on the wide range of Gram negative bacteria has not been achieved.

Immunoglobulins (gammaglobulins, antibodies)

In 1890 Emil von Behring published a scientific paper on the work on treatment of tetanus using antitoxin.

Subsequent studies have shown that antitoxin was immunoglobulins which became the starting point for passive immunotherapy in which immunoglobulins were prophylactically injected intramuscularly or subcutaneously against for example botulism, diphtheria, adder bite and snake

bite. The immunoglobulins all derived from immunized animals and were directed against toxins. Furthermore,immunoglobulins of human origin are applied in the prophylaxis of tetanus and hepatitis B.

It is well known that immunoglobulins play a role in the control of microbial infections. First, specific immunoglobulins bind to the surface of the bacteria whereby opsonization is possible which renders the bacterium in question more attractive to the phagocytes. It is furthermore well known that immunoglobulins on their own are not able to kill bacteria. It is necessary that an intact complement system is present which is activated by the immunoglobulins in order to kill bacteria. The fact that immunoglubulins are dependent on complement or phagocytes in the battle against bacteria has the effect that immunoglobulins are only seen to be capable of having an effect when they are injected whereby the immunoglobulins are mixed with serum and tissue fluid in which plenty of complement is present. This notion is supported by the fact that immunoglobulins do not have any effect against bacteria under in vitro conditions.

Compositions are described for local use comprising among other things immunoglobulins and lysozyme (EP 1068071 and US 4734279). These compositions undoubtedly have an effect against Gram positive bacteria due to their contents of lysozyme whereas Gram negative bacteria will not be affected by lysozyme nor lysozyme in combination with native immunoglobulins for local use. It is common knowledge that bacteria are equipped with proteolytic enzymes that are able to degrade immunoglobulins. Some bacterial enzymes have IgA1 as their specific substrate, the socalled post-proline endopeptidases. These measures are described in detail by Mogens Killian et al. in APMIS 104: 321-338, 1996. The IgG molecule that is not present in mucosal membranes is very sensitive to all sorts of proteases and it is inactivated after few minutes in most suspensions of bacteria.

Thus, repeated experiments in vitro have also shown lack of effect on Gram negative bacteria using the combination of lysozyme and native immunoglobulins.

The scope of the present invention is to assign an antimicrobial composition for local use without the side-effects and the disadvantages involved when using antibiotics and disinfectants. According to the invention this is achieved by a composition comprising a system consisting of a combination of lysozyme and glycosylated immunoglobulin.

The advantage of the present invention is that it has solved the problem of lysozyme penetrating the outer lipopolysaccharid membrane on Gram negative bacteria and thus created a possibility of degrading the peptidoglycan membrane resulting in bacteriolysis (Fig. 5).

The invention is based on in vitro experiments using Gram negative bacteria and their corresponding glycosylated immunoglobulins directed against antigens present on the surface of the bacteria.
Upon attachment of a glycosylated immunoglobulin on the surface antigen of the bacterial wall it was shown that the bacteria could be killed using lysozyme.
The possible explanation of this phenomenon is that the glycosylated immunoglobulin molecule (the antibody) binds to the surface antigen determinant of the bacterial wall whereby the characteristics of the surface of the bacterial wall is altered in such a manner that lysozomal activity on the underlying layer of peptidoglycan is possible.

It is a condition that the used immunoglobulins are intact and resistant towards bacterial proteases. This is achieved by glycosylating the FC fragment of the immunoglobulins. 10 g pooled immunoglobulins 77,0% IgG, 7,6% IgA and 15,4% IgM are dissolved in 25 ml 1M solution of glucose and incubated at 45°C. During the incubation the glucose will establish covalent bonds particularly to the Fc fragment of the IgG molecule which can be tested by demonstrating the loss of ability to bind complement. However, the ability of the glycosylated immunoglobulins to agglutinate is unchanged.
An analogous glycosylation has been observed in vivo in insufficiently insulin-regulated patients suffering from diabetes. A glycosylated immunoglobulin is extremely resistant to as well pancreatic proteases as bacterial proteases.

Non classified Gram negative rods and Gram negative cocci were used in laboratory experiments. The used lysozyme is extracted from hen's egg white and the immunoglobulins have been isolated from bovine milk and colostrum.

Experiment 1a
The suspension of bacteria (100.000 bacteria per ml) was incubated for half an hour in the presence of 5 mg per ml lysozyme at 37°C. Subsequent culturing on an agar plate did not show bacterial kill.

Experiment 1b
The suspension of bacteria (100.000 bacteria per ml) was incubated for half an hour in the presence of 5 mg per ml lysozyme + 40 micrograms per ml agglutinating native antibodies at 37°C. Subsequent culturing on an agar plate did not show bacterial kill.

Experiment 1c

The suspension of bacteria (100.000 bacteria per ml) was incubated for half an hour in the presence of 5 mg per ml lysozyme + 40 micrograms per ml agglutinating glycosylated antibodies at 37°C. Subsequent culturing on an agar plate showed 100 % bacterial kill.

Experiment 2a

The suspension of bacteria consisting of 2 different types of Gram negative bacteria are incubated for half an hour at 37°C in the presence of 5 mg per ml lysozyme + 40 micrograms per ml agglutinating native antibodies specific for one of the bacteria types. Subsequent culturing on an agar plate did not show bacterial kill.

Experiment 2b

The suspension of bacteria consisting of 2 different types of Gram negative bacteria are incubated for half an hour at 37°C in the presence of 5 mg per ml lysozyme + 40 micrograms per ml agglutinating glycosylated antibodies specific for one of the bacteria types. Subsequent culturing on an agar plate showed 100 % bacterial kill of the bacteria type to which the antibodies were directed against, and no effect on the bacteria that did not agglutinate with the used antibodies.

To correlate the in vitro experiments to the in vivo situation the suspension of bacteria was a growth medium such that the exogenous bacterial enzymes were present.

List of drawings

Figure 1:
Schematic drawing of the cell wall of a Gram positive bacterium.

Figure 2:
Schematic drawing of the cell wall of a Gram negative bacterium.

Figure 3:
Diagram showing the lysis of a Gram positive bacterium using lysozyme.

Figure 4:
Diagram illustrating that Gram negative bacteria are not affected by lysozyme alone.

Figure 5:

Diagram showing the lysis of a Gram negative bacterium using glycosylated immunoglobulin and lysozyme according to the invention.

Examples of compositions comprising the system according to the invention.

1) Composition for skin infections (gel)

Lysozyme	0.5%
Glycosylated immunoglobulins (pooled IgM, IgG, IgA)	0.4%
Resingel	99.1%
	100.0%

2) Composition for skin infections (cream)

Lysozyme	0.5%
Glycosylated immunoglobulins (pooled IgM, IgG, IgA)	0.4%
Cream base	99.1%
	100.0%

3) Composition for skin infections (wet tissue)

Lysozyme	0.5%
Glycosylated immunoglobulins	0.4%
Oil of peppermint	0.1%
Sterile water	99.0%
	100.0%

3) Lozenge for infections of mouth, throat and pharynx

Lysozyme	0.5%
Glycosylated immunoglobulins	0.4%
Flavour additives and tablet additives	99.1%
	100.0%

4) Tablet to be chewed for infections of the gastrointestinal tract

Lysozyme	0.5%

Glycosylated immunoglobulins	1.2%
Flavour additives and tablet additives	98.3%
	100.0%

Claims

1. Antimicrobial composition for local use on mucosal membranes and skin c h a r a c t e r i z e d by comprising a system consisting of a combination of lysozyme and glycosylated immunoglobulins with affinity to Gram negative bacteria.

2. Antimicrobial composition according to claim 1 c h a r a c t e r i z e d by the glycosylated immunoglobulins being of monoclonal or polyclonal origin.

3. Antimicrobial composition according to claim 2 c h a r a c t e r i z e d by the glycosylated immunoglobulins being of the classes IgM, IgG, IgA or dimer IgA.

4. Antimicrobial composition according to claim 1 c h a r a c t e r i z e d by the immunoglobulins originating from a biological fluid such as milk, whey, blood, plasma or serum.

5. Antimicrobial composition according to claim 1 c h a r a c t e r i z e d the lysozyme being native or conjugated.

Summary

Antimicrobial composition for local use on mucosal membranes and skin comprising a combination of lysozyme and glycosylated immunoglobulins with affinity to Gram negative bacteria.
Lysozyme is able to lyse Gram positive bacteria by degrading peptidoglycan of the bacterial cell wall (Fig. 3). The peptidoglycan of Gram negative bacteria is protected against the action of lysozyme by a layer of lipopolysaccharide (Fig. 4). When glycosylated immunoglobulins resistant to proteases bind to the cell wall of the Gram negative bacteria the surface of the lipopolysaccharide layer is altered in such a manner that lysozyme is able to degrade the underlying peptidoglycan layer, resulting in bacteriolysis (Fig. 5).

Figure 1

Cell wall of a Gram positive bacterium

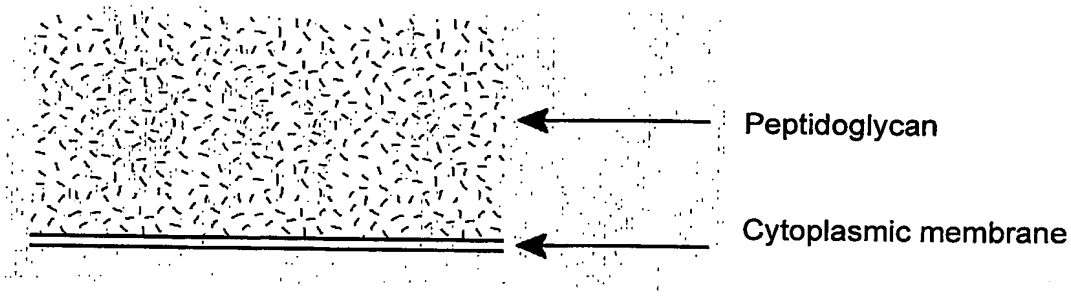


Figure 2

Cell wall of a Gram negative bacterium

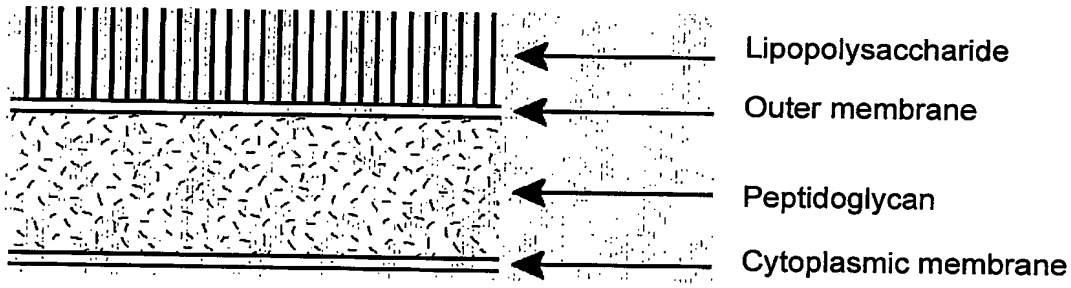


Figure 3

Gram positiv bacterium is lysed using lysozyme

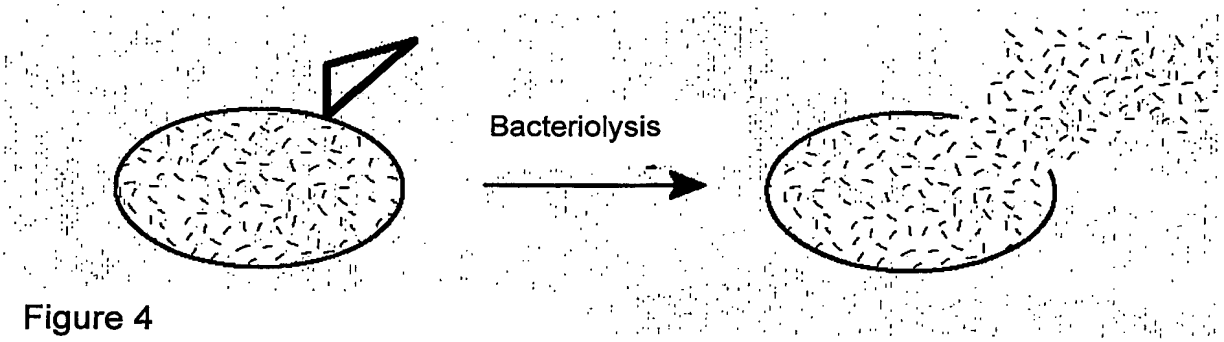


Figure 4

Gram negative bacteria cannot be lysed using lysozyme alone

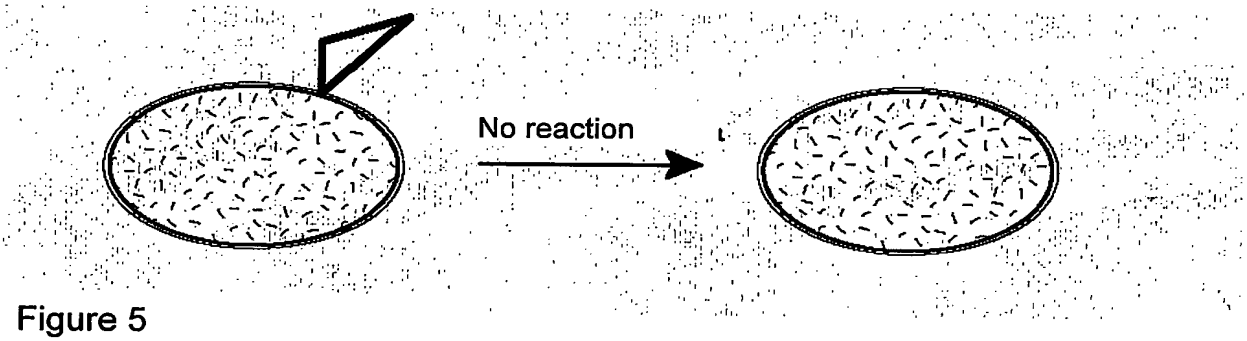
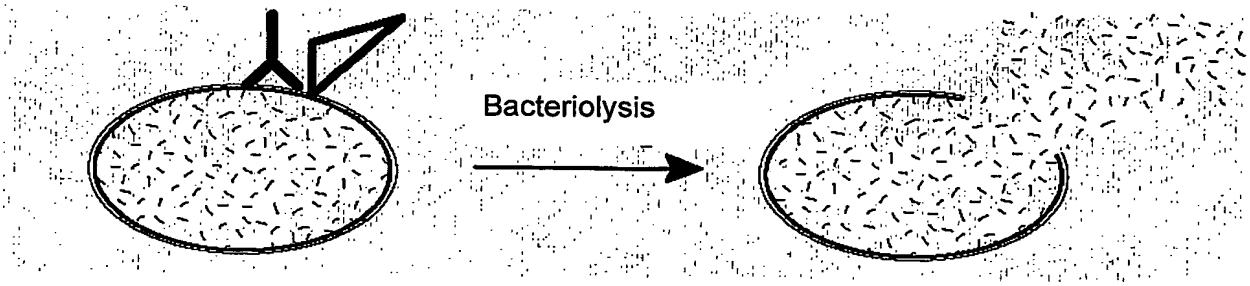
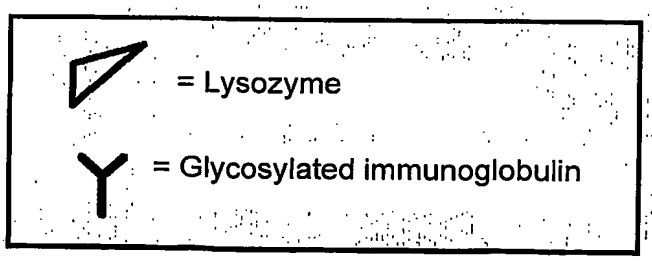


Figure 5

Gram negative bacterium is lysed using glycosylated immunoglobulin and lysozyme





Sheet No. 1

Box No. VIII (iv) DECLARATION: INVENTORSHIP (only for the purposes of the designation of the United States of America)

The declaration must conform to the following standardized wording provided for in Section 214; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (iv). If this Box is not used, this sheet should not be included in the request.

Declaration of inventorship (Rules 4.17(iv) and 51*bis*.1(a)(iv)) for the purposes of the designation of the United States of America:

I hereby declare that I believe I am the original, first and sole (if only one inventor is listed below) or joint (if more than one inventor is listed below) inventor of the subject matter which is claimed and for which a patent is sought.

This declaration is directed to the international application of which it forms a part (if filing declaration with application).

This declaration is directed to international application No. PCT/ DK03/00940 (if furnishing declaration pursuant to Rule 26*ter*).

I hereby declare that my residence, mailing address, and citizenship are as stated next to my name.

I hereby state that I have reviewed and understand the contents of the above-identified international application, including the claims of said application. I have identified in the request of said application, in compliance with PCT Rule 4.10, any claim to foreign priority, and I have identified below, under the heading "Prior Applications," by application number, country or Member of the World Trade Organization, day, month and year of filing, any application for a patent or inventor's certificate filed in a country other than the United States of America, including any PCT international application designating at least one country other than the United States of America, having a filing date before that of the application on which foreign priority is claimed.

Prior Applications: PA 2003 01858, filed January 2, 2003 in Denmark

I hereby acknowledge the duty to disclose information that is known by me to be material to patentability as defined by 37 C.F.R. § 1.56, including for continuation-in-part applications, material information which became available between the filing date of the prior application and the PCT international filing date of the continuation-in-part application.

I hereby declare that all statements made herein of my own knowledge are true and that all statements made on information and belief are believed to be true; and further that these statements were made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under Section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of the application or any patent issued thereon.

Name: Jens Richard Pedersen

Residence: Vejle, Denmark
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Citizenship: Danish

Inventor's Signature: Jens R. Pedersen
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 29.01.04
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

Name: Torben Richard Pedersen

Residence: Vejle, Denmark
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Citizenship: Danish

Inventor's Signature: Torben R. Pedersen
(if not contained in the request, or if declaration is corrected or added under Rule 26*ter* after the filing of the international application. The signature must be that of the inventor, not that of the agent)

Date: 29-01-2004
(of signature which is not contained in the request, or of the declaration that is corrected or added under Rule 26*ter* after the filing of the international application)

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (iv)".

Box No. VIII (ii) DECLARATION: ENTITLEMENT TO APPLY FOR AND BE GRANTED A PATENT

The declaration must conform to the standardized wording provided for in Section 212; see Notes to Boxes Nos. VIII, VIII (i) to (v) (in general) and the specific Notes to Box No. VIII (ii). If this Box is not used, this sheet should not be included in the request.

Declaration as to the applicant's entitlement, as at the international filing date, to apply for and be granted a patent (Rules 4.17(ii) and 51*bis*.1(a)(ii)), in a case where the declaration under Rule 4.17(iv) is not appropriate:

In relation to international patent application no. PCT/DK03/00940

Pedersen Medical A/S

is entitled to apply for and be granted a patent by virtue of the following:

An assignment dated 29 January 2004

from

Jens Richard Pedersen, Gludsmindevej 11, DK-7100 Vejle, Denmark

and

Torben Richard Pedersen, Højstrupvej 25, DK-7120 Vejle, Denmark

to

Pedersen Medical A/S, Jens Grøns Vej 15-17, DK-7100 Vejle, Denmark.

This declaration is made for the purposes of all designations except the designation of the United States of America.

☐ This declaration is continued on the following sheet, "Continuation of Box No. VIII (ii)".